Steven M. Ruben
Appl. No. 10/662,429

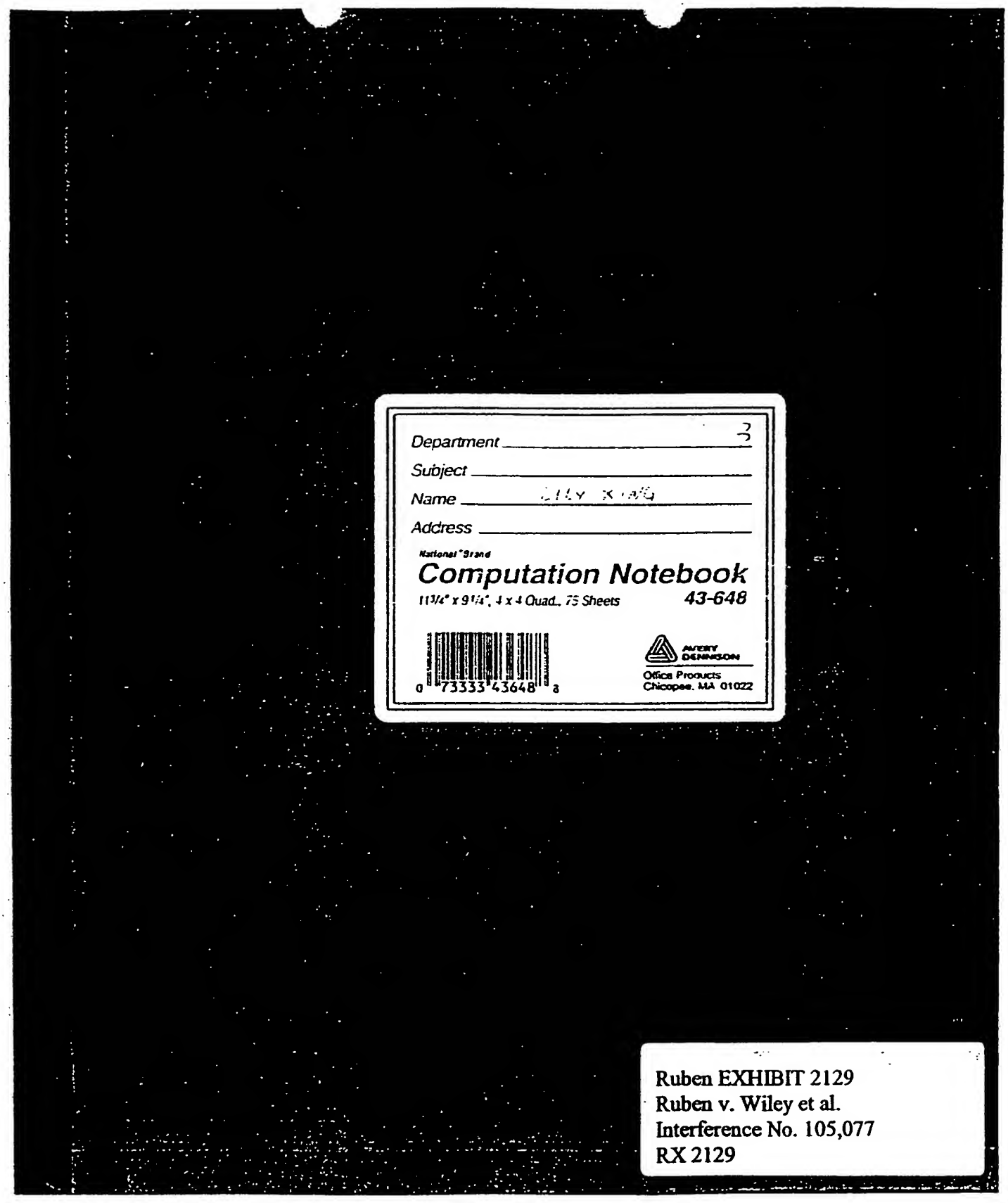
Department 3
Subject
Name
Address

National Brand
Computation Notebook
11 3/4" x 9 1/4", 4 x 4 Quad., 75 Sheets **43-648**

0 73333 43648 8

AVERY DENNISON
Office Products
Chicopee, MA 01022

Ruben EXHIBIT #129



Ruben EXHIBIT 2129
Ruben v. Wiley et al.
Interference No. 105,077
RX 2129

2-8-96 Test OD for construct primer

4616 Trail HindIII 3'
4617 " BamH2 5'
4618 " BamH1 5'
4619 HT4 HindIII 3'
4620 " BamH1 5'
4621 HoFmBo9 BamH1 5'
4622 " Asp718 3'

[illegible] Method [illegible] Print Vol

Results file: A:\WORK_RES

Method name: A:\DEFAULT

Assay type: General Ratio and Concentration
Formula setup: VIEW
Sampling device: One cell
Read average time: 0.50 sec

Units: ug/ml
Background Correction: [No]
Concentration: [No]
Peak Pick: [No]

Sample ID		abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	1:500
1	4616	0.0515	0.0330	1.5621	0.6402	0.9
2	4617	0.0400	0.0276	1.4471	0.6910	0.7
3	4618	0.0394	0.0260	1.5126	0.6611	0.6
4	4619	0.0484	0.0326	1.4830	0.6743	0.8
5	4620	0.0445	0.0291	1.5301	0.6536	0.7
6	4621	0.0613	0.0374	1.6401	0.6097	1
7	4622	0.0550	0.0391	1.4065	0.7110	0.9
8						

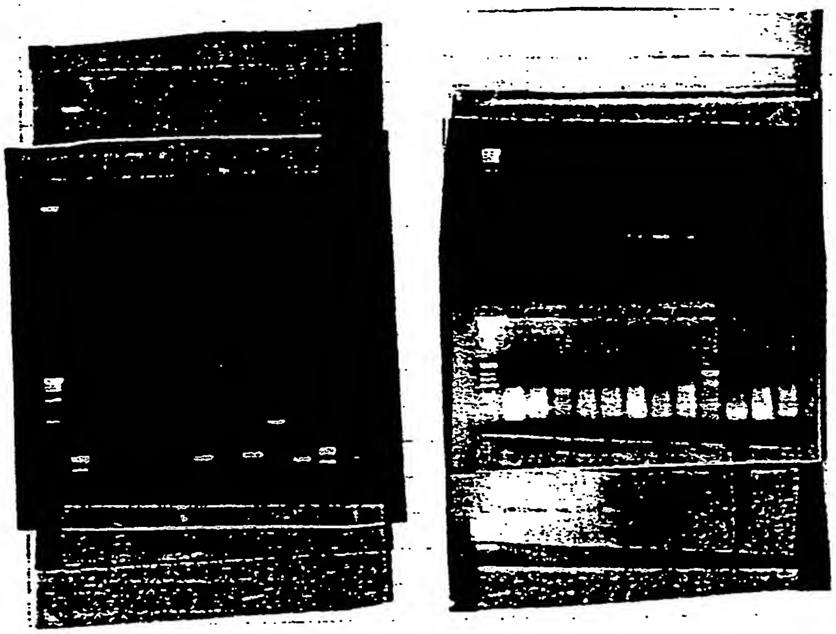
PCR for construct:

			Enzymes	Vector
1	HCAcub2	4319+4320 3'	BamH1 + Asp718	pA2
2	HCAcub2	4319+14351 3'	" BamH1 "	cHa
3	HTPA (Trail)	4616+4617 5'	HindIII + BamH2	pQE9
4	HTPA (Trail)	4616+4618 5'	HindIII + BamH1	pQE9
5	HT4	4619+4620 5'	HindIII + BamH1	pQE9
6	HoFmBo9	4621+4622 3'	BamH1 + Asp718	pA2
7	HTTBN61	4577+4578 3'	BamH1 + XbaI	A2-GP

2-8-96 PCR: for HTTBN61

use T3 primer + 4579 primer

Take 5ml Running 3% agarose gel for Southern Blot.



(1) HCQA
(2) HLGX
(3) HF
(4) HBm
(5) F. Brain
(6) Hela
(7) pine leck
(8) HCE
(9) HET
(10) HOF
(11) HPL
(12) HTX
(13) HE8
(14) HLH
(15) HPD
(16) HSOB
(17) HIKP
(18) HKL
(19) HFS
(20) HMG
(21) LV
(22) HMMA
(23) HTXA
(24) LV
(25) Hela

- Denature Buffer

labling HTTBN61 BamH1 specific primer → Buffer.

See oligo labling	1λ	(1ug) HTTBN61 primer
	5λ	10x T4 PNK Buffer at 30'
	1λ	γ-32P ATP
	1λ	T4 Kinase Enzyme (PNK)
	42λ	H_2O

37°C 30'

add 10ug of spiner DNA 100ml

5m NaPO4 PPT

primer BamH1

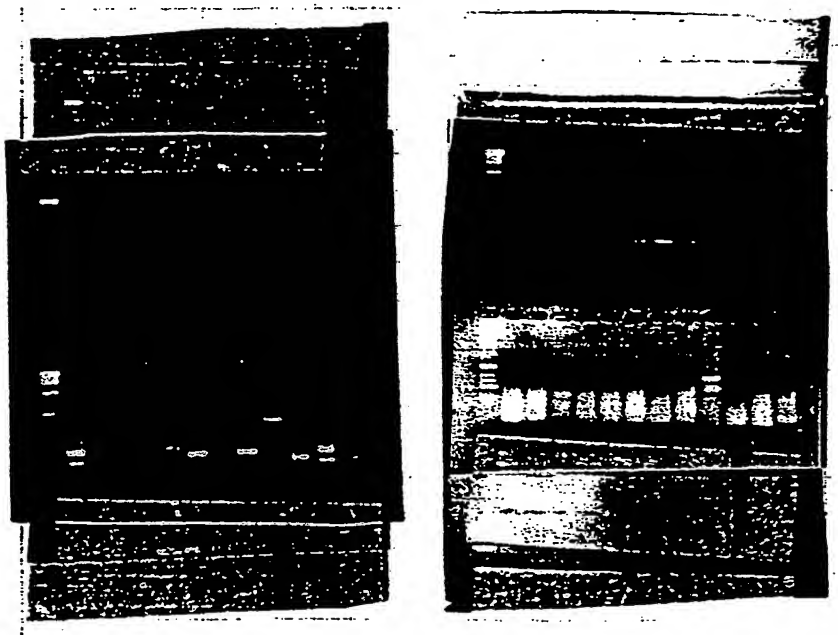
Conter: 1×10^5 / 200ml.

[signature] 2/9/96

3-8-96 PCR: for HTTBN61

use T3 primer + 4579 primer

Take 5ml Runing 3% agarose gel for Southern Blot.



(1) HLQA (17) HLKI
(2) HLGX (18) HKL
(3) HF (19) HFS
(4) HBM (20) HMG
(5) F. Brain (21) HMA
(6) Hela (22) HMA
(7) pinv (23) HTA
(8) HCE (24) LV
(9) HET (25) Hela
(10) HBF
(11) HPL
(12) HTX
(13) HES
(14) HLH
(15) HPD
(16) HSOS

- Denature Buffer
Take the gel put in 500ml Denature Buffer at RT 1 hr

= Neutralization Buffer
500ml shake the gel at RT. about 30'

= at 10xSSC transfer. o/weekend

- Make oligo probe: use HTTBN61 (4577) primer BamHI

Conter: 1×10^8/200ml.

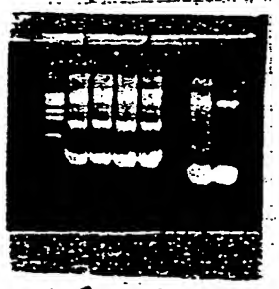
2/9/96

2-9-96 PCR for construct :

Running 2% low melting gel :

(17) HIKI
(18) HKL
(19) HFS
(20) LNG
(21) LNG
(22) HHMA
(23) HTXA
(24) LV
(25) HEIA

(MKEF)
1 HCAcub2
(NKEF)
2 HCAcub2
(FasL)
3 HTPA



(FasL)
4 HTPA
(FasLike)
5 HT4
6 HOFMB09
(TNFR)
7 HTTBN61

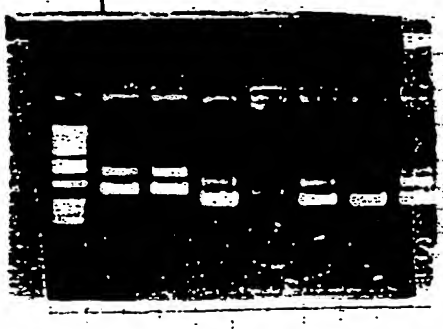
at 70°C 10'
phenol / ϕ X 3
EtOH ppt (c/weekend)

2-12-96 Degestion by Gunyner

30λ	
24λ	DNA + H2O
3λ	#2 Buffer
1λ	BamHI XbaI
1λ	HindIII Asp718

at 37°C incubate 2hrs
↓ phenol / ϕ X 3
↓ EtOH ppt

Take 3λ of each sample Running gel



2-12-96 ~~Submit~~ CSG7 CSG16 for sequencing
HPD012
use pRE 3' + 5' primer

2-12-96 Ligation to vector.

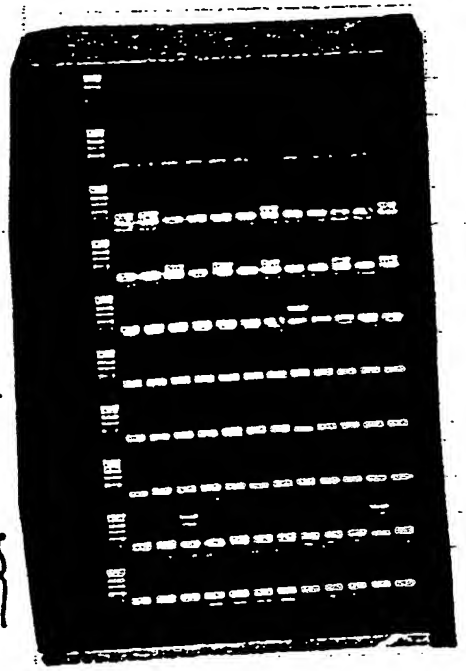
#1	HCAGWb2	pA2
2	"	cHO
3	HTPA	pRE4
4	HTPA	pRE4
5	HT4	pRE4
6	HOFIMBO4	pA2
7	HTTBWb1	A2-GP

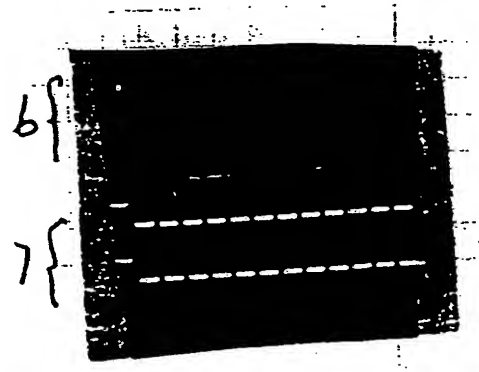
2-13-96 ~~transformation~~ to XL1Blue cell
2ml to 100ml XL1Blue cell
on ice 15'
42°C 1' add 1ml LB
37°C 1 hr
pour plates 500 λ. (LB+Amp plate)

2-14-96 ~~for sequencing~~:
CSG
Wash Southern Blot. at RT 2 time
Exp. 4 hrs

2-14-96 pick up single clones in 100ml LB + Amp

make PCR Reaction:





grow up DNA

#2 — 12

#3 — 8

#7 — 1

2-15-96 all constructed PCR primer use vector primer

made DNA prep:

BECKMAN DU-600

Date:

Nucleic Acid ReadSamples	Method			
Hi Amba				2.6
HTPA 3			1.5330	2.5
HTPA 7		1.0075	0.5532	2.5

2-14-96 pick up 12 clone from HTTBU61 PCR product

use Enlgland Buffer ppt.

-Clean: add 100ml Enlgland Buffer

at RT 10'

centrifuge 10'

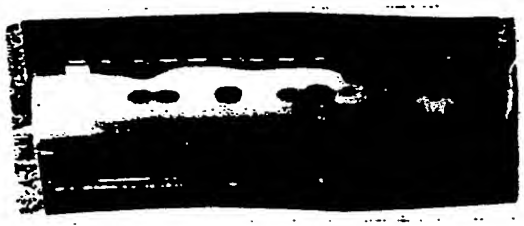
70% EtOH wash

Resuspened in 10ml of H_2O

Running on the gel to check the DNA

Submit for sequencing.

primer use HTTBU61 RPR3

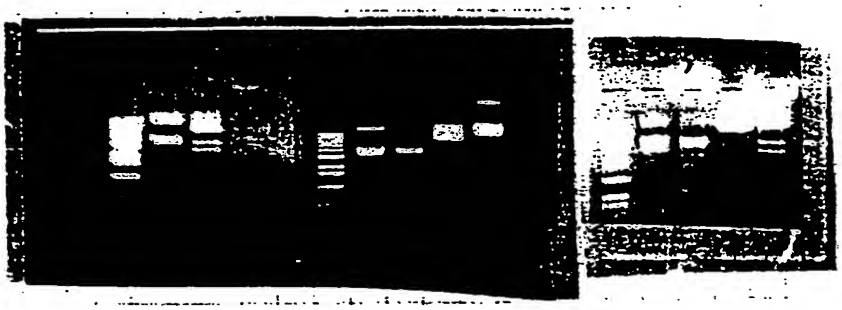


1 — 8 10 — 12 use 4ml of DNA

#9 use 1 ml of DNA.

2-15-96 Degestion

1K	HCAEBU62 uncut 2	HTPA uncut 3	HTTBU61 uncut 7



2-15-96 ligation again

#1	HeAcub2	pA2
#3	HTPA (Trail)	pQE9
#4	HTPA (Trail)	pQE9
#5	HT4	pQE9
#6	HOFmB09	pA2
#7	HTTBN61	A2-GP

20λ

3λ	DNA insert
3λ	vector
4λ	5x Ligation buffer
1λ	T4 DNA ligase
9λ	H2O

RT 3.5 hrs.

2-16 PCR for construct : primer Enzyme Vector

(1)	HSABH13	4335 + 4336	[redacted]	pA2
(2)	HTXE133	4292 + 4293	[redacted]	pA2
(3)	HOFmB09	4621 + 4622	[redacted]	pA2
(4) ✓	HTTBN61	4577 + 4578	[redacted]	A2-GP
(5) ✓	HPDD012	4581 + [redacted]	[redacted]	pQE9
(6) ✓	HTPA (Trail)	4616 + 4617	[redacted]	pQE9
(7) (for sequencing number) (HTPAT) ←	HTPA (Trail)	4616 + 4654	[redacted]	pQE9
(8)	HT4	4619 + 4620	[redacted]	pQE9
(9)	FASL (HFFCD16)	4657 + 4656	[redacted]	pQE9

2/20/96

2-20-96 Runing 2% low melting gel: (for construct)

cut down insert 70°C 10'

phenol/φ x-3

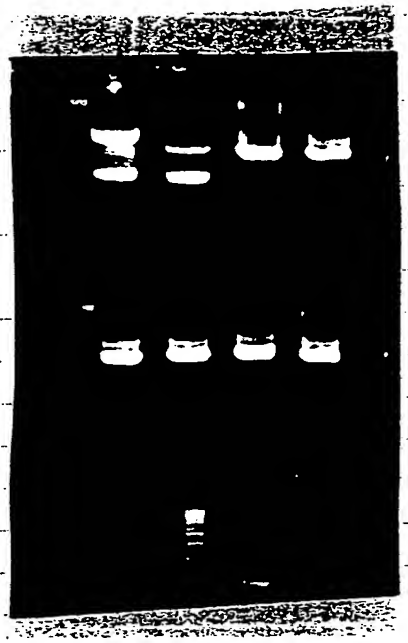
EtOH ppt o/n

↓

2-21-96 resuspend pllet in 25λ H_2O

Degestion by Enzymes

	30λ
Dd H_2O	25λ
B Buffer	3λ
Enzymes	1λ
"	1λ



See 2/16/96

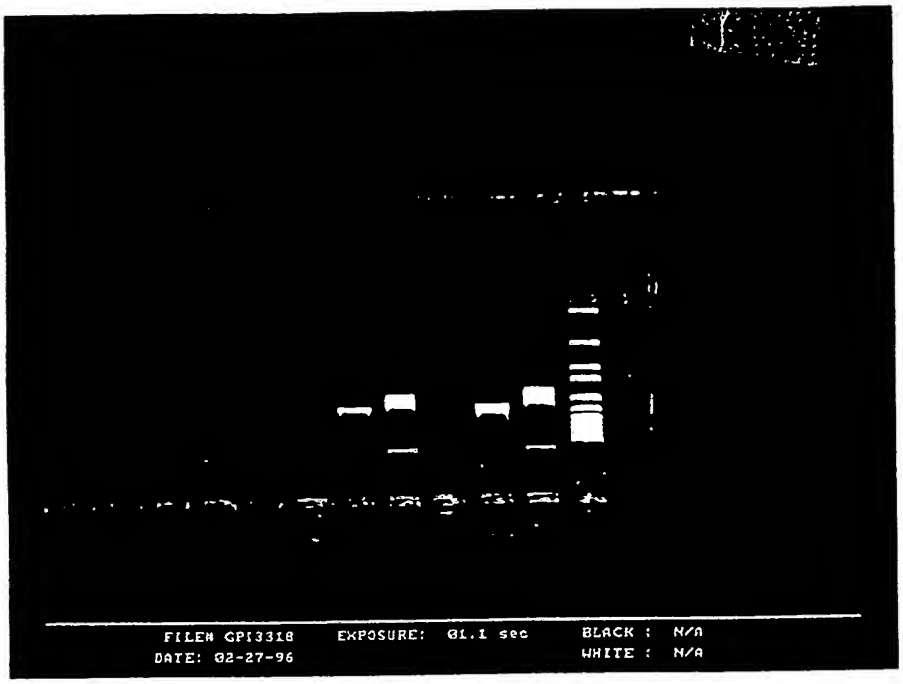
37°C incubate 2hrs Sma I + Bgl II at 25°C incubate

↓ after 2hrs Take 3λ of each sample Runing on the gel:

vector

2-20-96 Runing 2% low melting gel: (for construct)

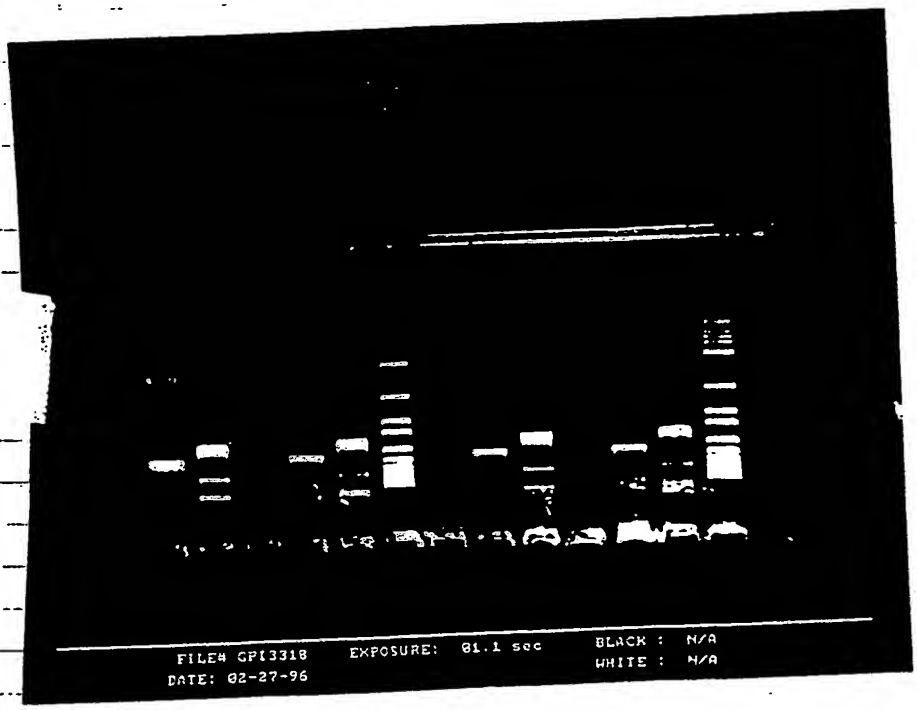
a dark incent. 70 c. 10'



See 2/16/96

5°c incubate

after 2hrs Take 3λ of each sample Runing on the gel:



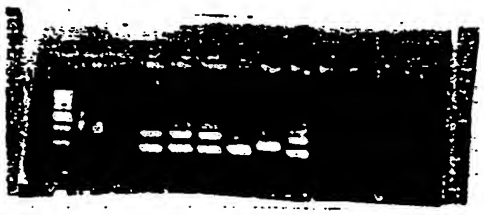
2-21-96 Degestion pQEq-vector
cut by BamHI + HindIII.

2/16/96

pQEq
Take 5ul Running gel ← BamHI + HindIII.

2-22-96 PCR fr: constructed See page 125

100λ PCR pr.
150λ Promega Buffer (direct purification Buffer)
1ml PCR Preps DNA purification Resin)
rt RT incubate 2' pass column
80% isopropanol wash x2
50ml H2O
Take 5ul Running on the gel.



Take 45λ degestion by enzymes:
after degestion: phenol/φ x3 EtOH ppt. resuspend in 10μl H2O
Ligation into vector:

1or	
2λ	5x Ligation Buffer
1λ	Vector
6λ	DNA
1λ	T4 DNA Ligase

RT 5hrs

2-23-96 PCR:

#4 HTTBNb1 — use pA2 3' + 5' vector primer

{5, 6, 7} — use pUC5 + 3' vector pr[imer]

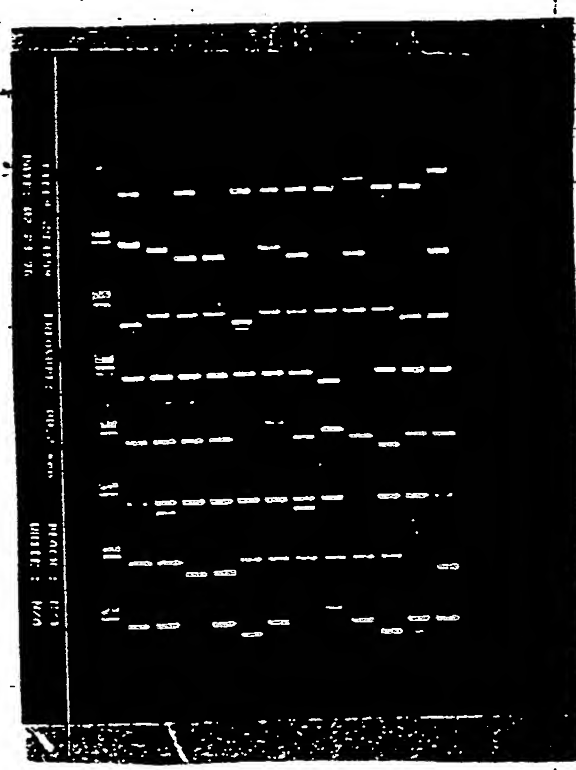
clone:

4 — #3 #5 #9 #14

5 — #2 #3

6 — #1 #2

7 — #1 #2



[redacted]-25-96 grow up DNA

2-26-96 make DNA prep:

Sample ID		abs 260.0 nm	abs 280.0 nm		260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
{	4-3	0.2317	0.1364	HTTBNb1	1.6990	0.5886	5.8
	4-5	0.1649	0.0933	A2-GP	1.7679	0.5656	4.1
	4-7	0.0704	0.0403		1.7461	0.5727	1.8
	4-14	0.0805	0.0427		1.8850	0.5305	2.0
5	5-2	0.0536	0.0271	HPmeil pu5	1.3729	0.5069	1.3
6	5-3	0.0877	0.0472		1.8594	0.5378	2.2
7	6-1	0.0730	0.0381	HTPA	1.9152	0.5221	1.8
8	6-2	0.0651	0.0339		1.9186	0.5212	1.6
9	7-1	0.0798	0.0415	HTPAS	1.9239	0.5198	2.0
10	7-2	0.1008	0.0532		1.8951	0.5277	2.5
11	6	0.0623	0.0383	465G	1.6278	0.6144	1-7/1
12	57	0.0761	0.0474	465	1.6068	0.6223	1.3.1-3
13							

Submit for sequencing

HTTBNb1 use pA2 3'+5'

Hpmeil

HTPA

HTPAT

2-26-96 PCR for construct.

#1 HSABH13 > use pA23'+5'
#3 HOFMBOJ
#8 HT4 — use pA65'+3'

this new CSG16 NCOL 5' primer.

- PCR for CSG16 5'15296 + 14813' NCO1 + HindIII pA260
HTXE133 4293 + 4293
(HNF1016) FASL 4656 + 4657

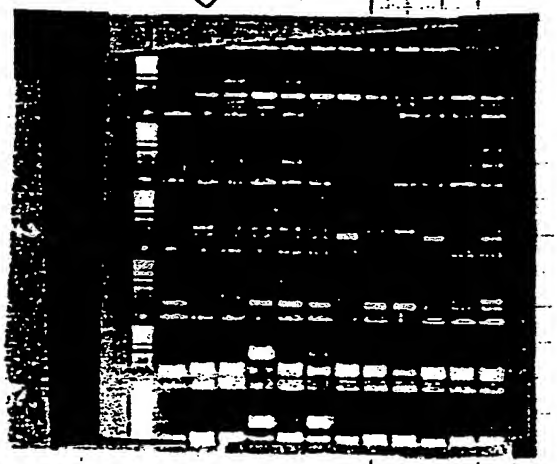
2-27-96

HSABH13 ←
#3 A20

HOFMBOJ ←

grow up DNA
O/N

HT4 ←
#4 #16



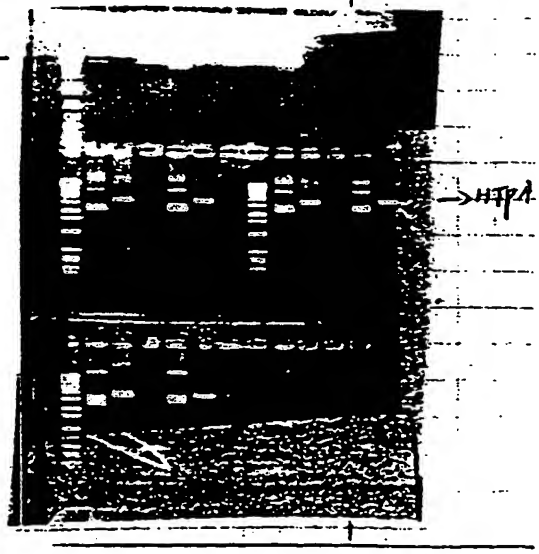
- Digestion 2/26/96 DNA prep. Runing 1% agarose gel:

HTTBN61 ←

HPOOOOO12 ←

HTPA BamH3 ←

→HTPA



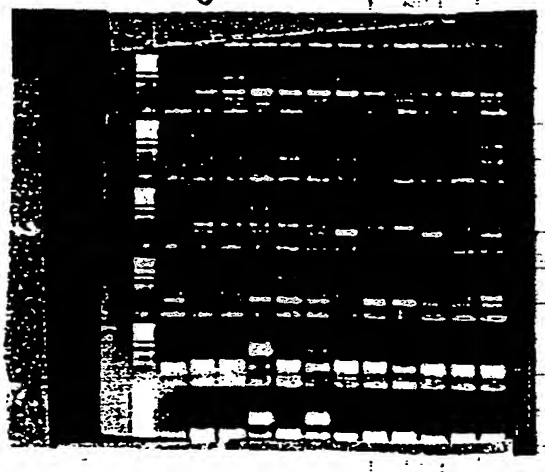
2-26-96 PCR for construct.

#1 HSABH13 > use pA23'+5'
#3 HOFMB09
#8 HT4 — use pA65'+3'

this new CSG16 NCO1 5' primer

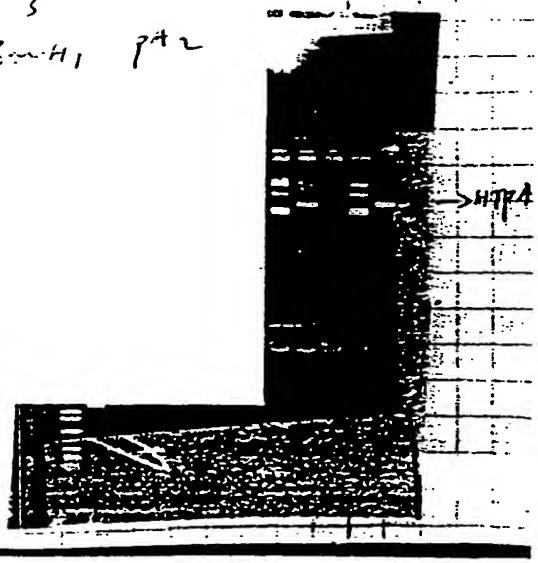
- PCR for CSG16 5'1529+1481 3' NCO1+HindIII pA2Bo
HTXE133 4293+4293
(HNF1016) FASL 4656+4657

2-27-96



grow up DNA O/N

- Digestion 21+10L mini prep ... 01
5 3
HCACU02 BamHI + BamHI pA2



2-27-96

12841	pA2 / A2GP 3'
1474	pA2 5'
2785	A2GP 5'

2-28-96

Results file: A:\WORK_RES
Method name: A:\DEFAULT

Assay type: General Ratio and Concentration
Formula setup: VIEW
Sampling device: One cell
Read average time: 0.50 sec

Units: ug/ml
Background Correction: [No]
Concentration: [No]
Peak Pick: [No]

1:500

Sample ID		abs 250.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
1	5	0.3443	0.0233	1.9006	0.5262	1:1
2	4	0.0736	0.0398	1.8498	0.5406	1:8
3						

Submit for sequencing:

HSABAH3 #3 pA2 3' +5'

HTU #4 pA2 3' +5'

2-28-96 (HLAIHC58) TAFReceptor full

Forward
Reverser
HTABLE1 RPR1 RPO2
RPR3 R4
RPO1 F5
F3 R5
R3
F4

Repeat Sequencing

Reverser >add 1% DMSO
F3

2-29-96

2-29-96 PCR for HTXEL33 pAz → use pAz 3'+5'

HTTBN61 A2-GP → use pAz3'+ A2-GpS'

FABL (HNF1α1b) pRE9 → pRE 3'+5'

CSG16 p026a

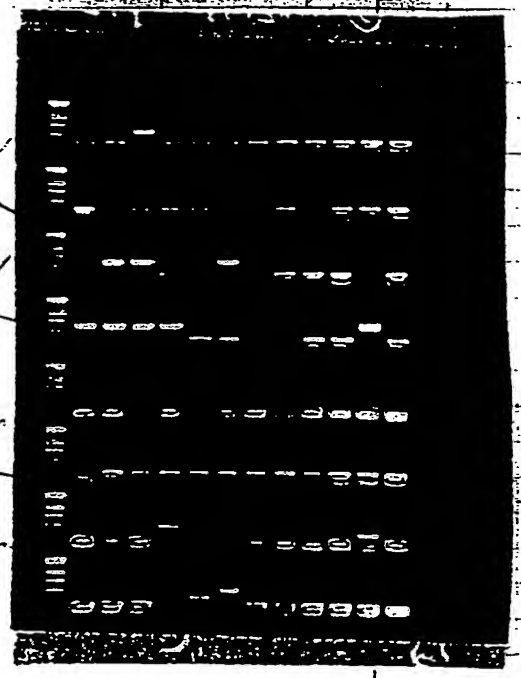
grow up DNA o/n

HTXEL33
pickup #3

HTTBN61
#2 #14

FAL(HNF1α1b)

CSG16
#4 #16 #18



3/1/96 make DNA prepr:

Submit for sequencing:

HTXEL33 pAz 3'+5'

HTTBN61 A2-GP 3'+5'

CSG16 pRE3' +3'

Submit HTTBN61 for sequencing

HUMHNF3B: Reverse add 1% DMSO
F3

Nucleic Acid
ReadSamples Method SaveClear Print Q

Results file: A:\WORK_RES Method name: A:\DEFAULT

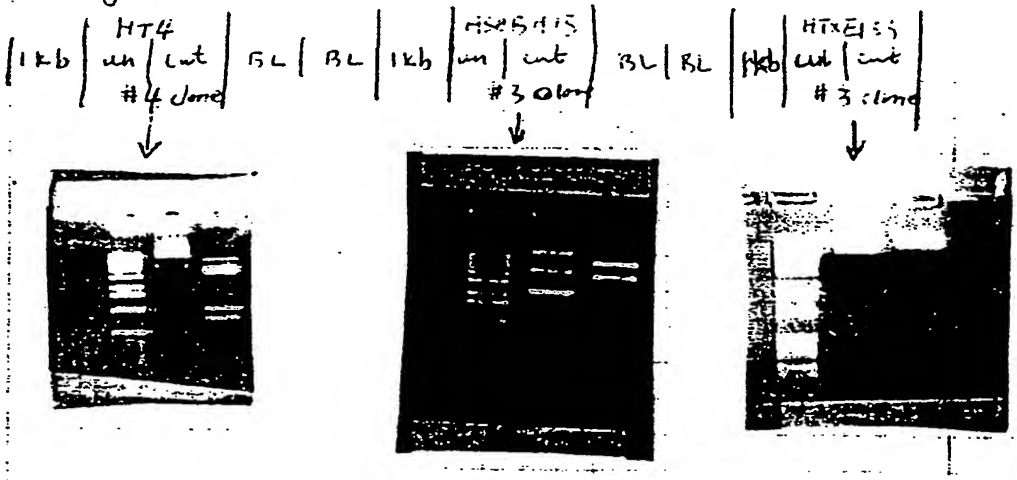
Assay type: General Ratio and Concentration Units: ug/ml
Formula setup: VIEW Background Correction: [No
Sampling device: One cell Concentration: [No]
Read average time: 0.50 sec Peak Pick: [No]

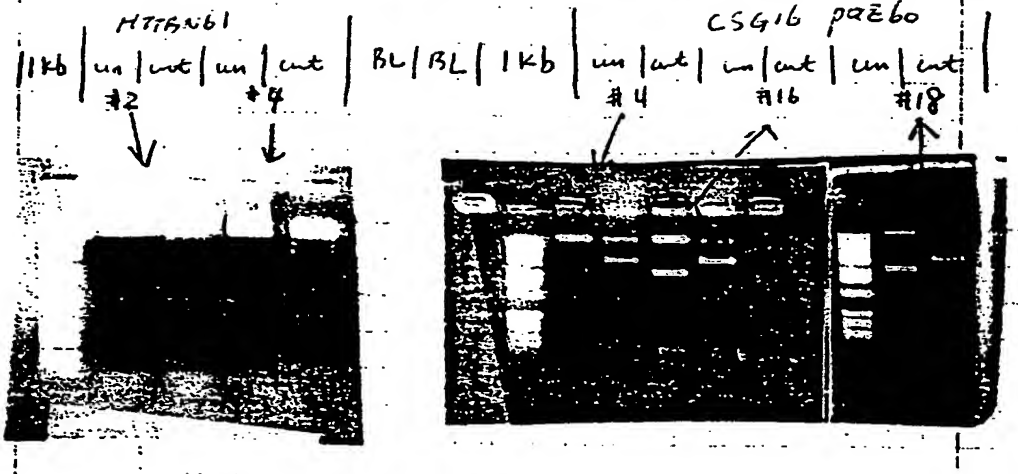
Sample ID	abs 260.0 nm	abs 280.0 nm		260.0 nm / 280.0 nm	260.0 nm / 280.0 nm	1:50
1	0.0438	0.0234	#3	1.8724	0.5341	1.1
2	0.0794	0.0412	#2	1.9274	0.5188	2
3	0.0443	0.0245	#14	1.8070	0.5534	1.1
4	0.0171	0.0078	#4	2.1931	0.4560	0.4
5 CSG16	0.0358	0.0184	#16	1.9322	0.5176	0.9
6	0.0244	0.0130	#18	1.8756	0.5332	0.6
7						

5-4-96 Degestion DNA (Seq-ge 151)

				Buffer
(1)	HT4	pAE9	BamHI + HindIII	B
(2)	HSABH13	pAz	BamHI + Asp718	B
(3)	HTXE133	pAz	SamI + Asp718	A
(4)	HTTBN61	Az-GP	BamHI + xbaI	A
(5)	CSG16	pQE60	NcoI + HindIII	B

Running 1% agarose gel :





3-5-96 Digestion for HTTBsiBI/A2-4p #9 clone

cut o/n.

transformation to JM15 cell.

Hpo0012 #3 clone

HTPA#03504 #2 clone (short)

HTPA#03504 #2 clone (long)

HT4 (from ligation).

pour plate (LB+A+K)

3-6-96 grow up clones at LB+A+K medium 37°C o/n.

(for protein induction)

grow up: Hpo0012 #3 1 — 4

HTPA (short) #2 5 — 8

HTPA (long) #2 9 — 12

CSG6 13 — 15

CSG7 16 — 17

3-7-96 Take 30λ to 3ml LB+A+K.

37°C 2.5 hrs

add 30λ IPTG to 3ml

37°C 3 hrs

3-7-96 Take 800λ spin down. wash 1

add 15λ of H_2O
15λ 2x SDS loading buffer
mix boil 5'

Running 10% SDS page:

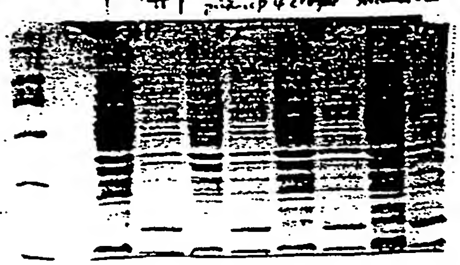
#1 gel: | m | BL | 1 | 1+ | 2 | 2+ | 3 | 3+ | 4 | 4+ |

#2 gel: | m | 5 | 5+ | 6 | 6+ | 7 | 7+ | 8 | 8+ | BL |

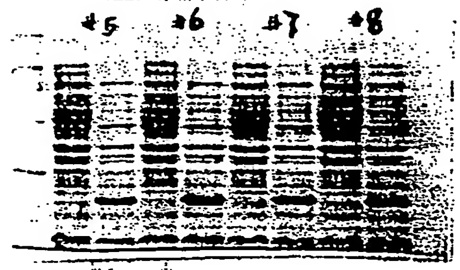
#3 gel: | m | 9 | 9+ | 10 | 10+ | 11 | 11+ | BL | 12 | 12+ |

#4 gel: | m | 14 | 14+ | BL | 15 | 15+ | 16 | 16+ | 17 | 17+ |

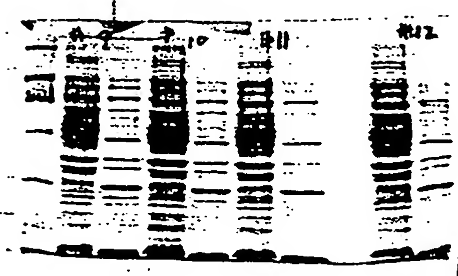
#1 HPDD012 #1/transformants



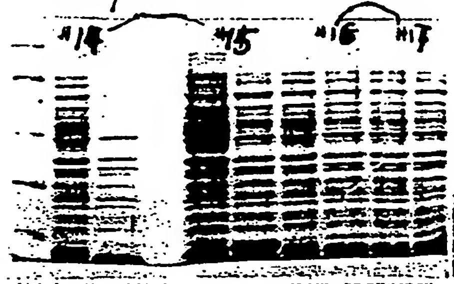
#2 HTPA(trail short) #2/transformants



HTPA(trail-long) #2/transformants
#3



CS66/p839 #4 CS67/p839

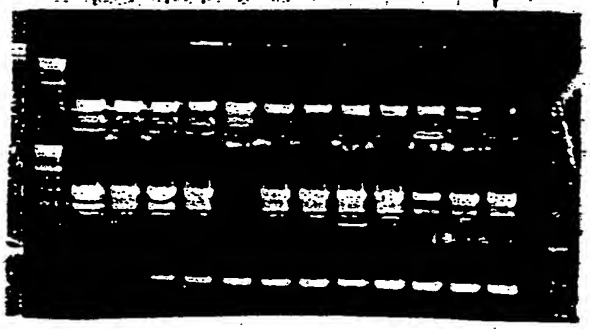


3-8-96 freezing HPDD012/p839 BamH1+HindIII #1 2.3.4
HTPA (trail short)/p839 BamH1+HindIII #5.6.7 8
HTPA (trail long)/p839 BamH1+HindIII #9 10 11 12

3-7-96 PCR for HTU (constant) in miS Cell

use 461j +462u specific primer. Total 36 clone

3-8-96 Running 1% agarose gel:



pickup # 13 #15

(protein induction)

3-9-96 grow up HT4 #13 #15 O/N

LB + A + K medium

3/14/96

→ Jim Ni: Title HPODV12

HTPA - long #1 clone

HTP4 - short #1

3/15/96

3-11-96 protein induction

↓

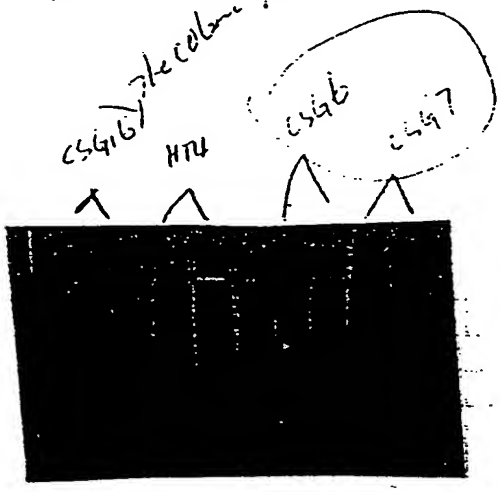
Take 30A + 0 3rd LB/AMP medium

↓ 37°C 2.5 hrs

↓ add 30A of IPTG (~~[redacted]~~)

↓ 37°C 3hrs

Running 15% SDS page: the colony not good.



— Repeat 3/7/96 gel

~~Repeat~~

Repeat csg16 HTH ccr2 > construct. See 3-11-96

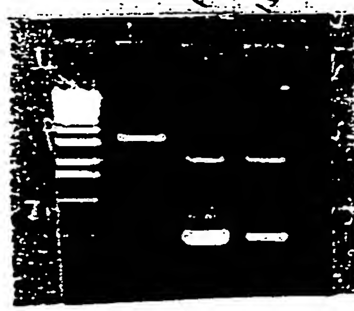
5-11-96 PCR for CSG16 Nco1 + BamH1 / pA2

(HCQAJ72) ② CSG16 15296 Nco1 5' + 12121 BamH1 3' (HCQAJ72)
① CSG16 15296 Nco1 5' + 1481 3' HindIII 3' (")

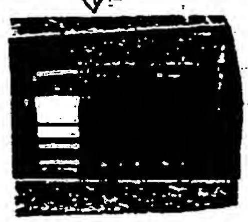
5-12-96 after PCR Take 5λ Running on the gel:

1529b+1481 ①

1529b+12121 ②



↓ Take 100λ of PCR
100λ promega buffer (kit)
1 ml PCR DNA Resin
RT 1'
↓ pass column wash x2 80% isp
↓ add 100λ of H2O
↓ Take 5λ Running on the gel

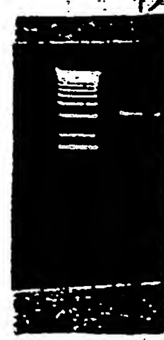


digestion: Take 43λ of PCR insert
5λ of B-Buffer
1λ Nco1
1λ BamH1
37° incubate 2 hrs
loading all sample on the gel:
↓
→ geneclean
↓
after geneclean
Running on the gel

after Degestion Running on the gel

cut down insert

Degestion pQE60 Nco1 + BamH1
after O/N Take 3λ Running on the gel



pQE60 Nco1+BamH1

the DNA gel in Vectors

3-13-96 HTH continued:

Digestion BamHI + HindIII

37°C 2hrs

↓ phenol X 2

↓ chcl3 X 1

↓ EtOH ppt

↓ resuspend in 10ml H_2O

— Ligation: to pQE9 vector (BamHI + HindIII)

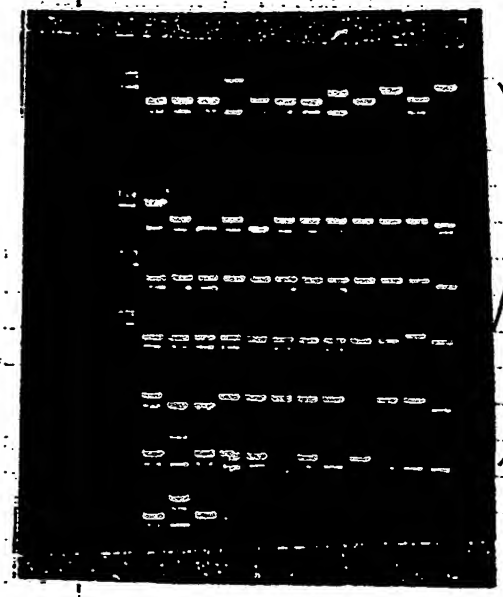
— Transformation to M15 cell:

— 37°C O/N

3-14-96 PCR for CSG16/pQE60 (1529b + 121=1)
NCOI + BamHI

HTH4472/pQE9 BamHI + HindIII

use pQE 3' +5' primer



pickup clone

CSG16 #1 2 3 4

HTH 5.6.7.8

SUPERVISOR Patrick J. Fuller

DATE 03/14/96

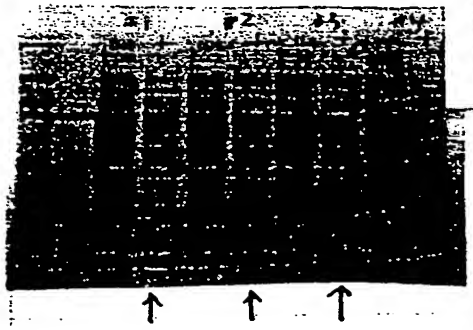
3-15-96 CSG16 protein induction.
HT4

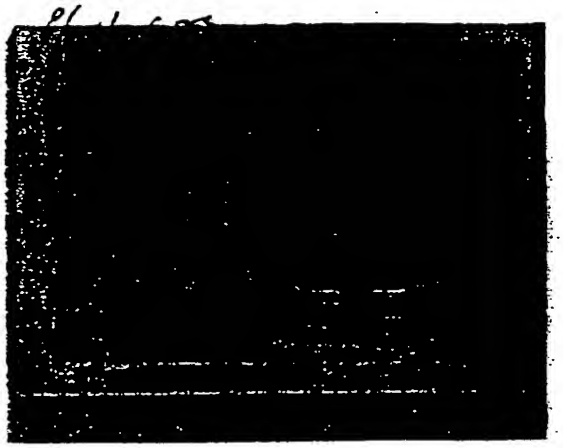
CSG16 1—4 clone
HT4 5—8 clone

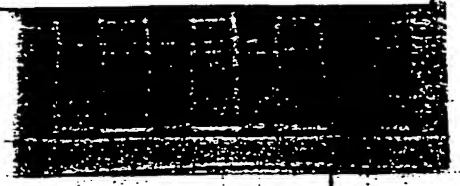
#1 gel: |m| BL | 1 | 1+ | 2 | 2+

#2 gel: |m| 5 | 5+ | 6 | 6+ | 7 | 7

#3 gel: CSG16/pQE60







clone from Jim Ni 3/26/96

3-15-96 freezing-go CSG16/pQE60 NCO1 + BamHI #1 #2 clone
HT4cc72/pQE9 BamHI + HindIII #5 #6 #7 #8 clone.

3-17-96 grow up CSG16 #1, 2
HT4cc72 #5 #6 clone for DNA.

3-18-96 Do DNA prep: for CSG16 #1, 2
HT4cc72 #5, 6

Assay type: General Ratio and Concentration
Formula setup: VIEW
Sampling device: One cell
Read average time: 0.50 sec

Units: ug/ml
Background Correction: [No]
Concentration: [No]
Peak pick: [No]

Sample ID		abs 260.0 nm	abs 280.0 nm	1:500	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
1	CSG16 #1	0.0406	0.0209		1.9434	0.5146	1 ug/ml
2	CSG16 #2	0.0330	0.0176		1.8681	0.5353	0.8
3	HT4 #5	0.0754	0.0406		1.8565	0.5387	1.9 dilution 0.9 ug/ml
4	HT4 #6	0.0341	0.0161		2.1179	0.4722	0.9
5	pQE60A	0.0656	0.0359		1.8287	0.5466	1.6
6							